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ISOLATION AND PARTIAL CHARACTERIZATION OF PLASMA MEMBRANES FROM--ETC(U)
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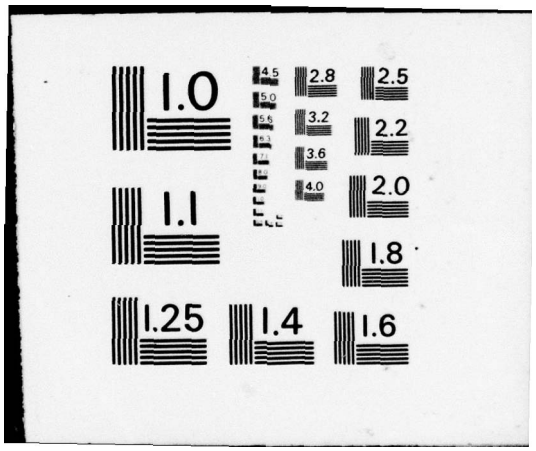
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Plasma membranes were isolated from the livers of control and Streptococcus pneumoniae-infected rats. Purity of the fractions was judged by electron microscopy and by the use of enzyme markers for microsomes (glucose-6-phosphatase), mitochondria (glutamate and malate dehydrogenase), and lysosomes (acid phosphatase). Plasma membranes from infected cells banded at the same sucrose density as plasma membranes from uninfected cells. Moreover, equivalent amounts of plasma membranes could be isolated from control and infected rat livers.

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There were, however, significant alterations in the enzyme complement of the plasma membrane following infection. 5'-Nucleotidase activity was significantly decreased while alkaline phosphatase activity was significantly increased. Kinetic analysis demonstrated that only the V_{max} and not the K_m of these two enzymes was changed, suggesting that altered affinity of the enzymes for substrate was not the mechanism responsible for the observed alterations. No change in the mitochondrial enzyme markers was observed following infection but the specific activity of microsomal glucose-6-phosphatase decreased significantly. Possible explanations for the observed alterations are discussed.

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Running Title: PLASMA MEMBRANES AFTER PNEUMOCOCCAL SEPSIS

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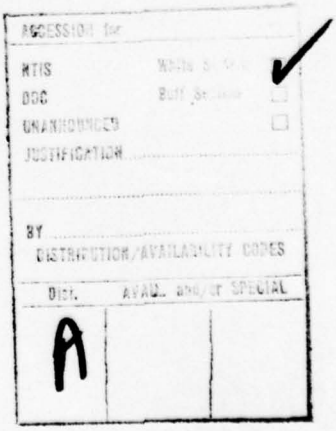

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ABSTRACT

Plasma membranes were isolated from the livers of control and Streptococcus pneumoniae-infected rats. Purity of the fractions was judged by electron microscopy and by the use of enzyme markers for microsomes (glucose-6-phosphatase), mitochondria (glutamate and malate dehydrogenase), and lysosomes (acid phosphatase). Plasma membranes from infected cells banded at the same sucrose density as plasma membranes from uninfected cells. Moreover, equivalent amounts of plasma membranes could be isolated from control and infected rat livers. There were, however, significant alterations in the enzyme complement of the plasma membrane following infection. 5'-Nucleotidase activity was significantly decreased while alkaline phosphatase activity was significantly increased. Kinetic analysis demonstrated that only the V_{max} and not the K_m of these two enzymes was changed, suggesting that altered affinity of the enzymes for substrate was not the mechanism responsible for the observed alterations. No change in the mitochondrial enzyme markers was observed following infection but the specific activity of microsomal glucose-6-phosphatase decreased significantly. Possible explanations for the observed alterations are discussed.

INTRODUCTION

Streptococcus pneumoniae infection induces marked alterations in systemic host metabolism which involve the liver. There is an increased uptake of zinc (18) and amino acids (30) into liver and increased acute phase globulin synthesis by liver (Beisel, In CRC Handbook of Nutrition and Foods, in press, 1976). Plasma glucagon and insulin are also elevated during this infection and affect hepatic carbohydrate metabolism (21, 22, 33). The plasma membrane may, therefore, play an important role in altered hepatic metabolism during this infection. There are indications that infection alters the enzyme complement (33, Canonico, Ayala, Rill and Little, Am. J. Clin. Nutr., in press, 1976), and surface properties (9, 13, 27) of several cell types. It was therefore of interest to study whether S. pneumoniae infection, which is known to induce decreases in other intrahepatic organelles (3), would also effect alterations in the plasma membrane.

MATERIALS AND METHODS

Chemicals. Nucleotides were obtained from P-L Biochemicals (Milwaukee, Wis.). All other chemicals were of analytical grade and obtained from commercial sources.

Animals. Male albino rats (150-200 g) of the Sprague-Dawley strain (Crl:COBS^{R} CD^{R} (SD) BR) were obtained from Charles River and were maintained on stock Purina Lab Chow and tap water ad libitum. All rats were acclimatized to a 12-h day-night cycle for at least 7 days prior to experimentatation in order to eliminate circadian variations. Rats were inoculated subcutaneously (s.c.) with 3 to 6 x 10^5 heat-killed (control) or virulent (infected) S. pneumoniae, serotype I, A5 strain (USAMRIID) organisms. All rats were fasted during the experiment and killed 20 or 40 h after inoculation at 8:30 A.M., a time corresponding to the midpoint of the night cycle.

Analytical procedures. Inorganic phosphate (Pi) was determined by the method of Chen et al. (4). Total phosphate was determined by the method of Ames and Dubin (1) and protein by the method of Lowry et al. (15) using bovine serum albumin as a standard. Concentrations of glucose-6-phosphate (G-6-P) and β-glycerolphosphate were determined by assaying total phosphate. Nucleotide concentration was determined by the measurement of its absorbance as described by Bock et al. (2).

Cell fractions. Plasma membranes (Fig. 1) were isolated by a modification of the method of Ray (19). For both control and infected animals 4 g of liver were excised and placed in ice-cold buffer (0.25 M sucrose containing 0.5 mM $CaCl_2$ and 1 mM Tris-Cl, pH 7.5). The livers were minced with scissors and then homogenized 8 strokes in a Dounce homogenizer

containing 40 ml of buffer. This homogenate was filtered through four layers of sterile gauze, diluted to 80 ml with buffer, and centrifuged at 1000 x g for 10 min in a Sorvall RC2-B refrigerated centrifuge (Ivan Sorvall, Inc., Norwalk, Conn.). The supernatant from this step was used for the preparation of microsomes and mitochondria. (See below). The pellet was washed twice by suspension and recentrifugation at 1000 x g for 10 min in 40 ml of 1 mM EGTA. The final pellet was suspended in 2 to 4 ml of 1 mM EGTA and adjusted to 48% sucrose (wt/wt) by the addition of 2.45 M sucrose. This sucrose suspension was divided equally into two Beckman SW 27 rotor tubes (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) and layered with 10 ml of 45% sucrose (wt/wt), then 10 ml of 41% sucrose (wt/wt), and the tubes were filled with 37% sucrose (wt/wt). The tubes were centrifuged at 131,000 x g for 2 h in a Beckman Model L2-65B ultracentrifuge. The plasma membrane fraction, which bands at the 37 - 41% sucrose interface was removed with a syringe, diluted with deionized water to 8% sucrose (wt/wt), sedimented by centrifugation at 10,000 x g for 10 min and finally suspended in 2.0 ml of deionized water.

Preparation of microsomes and mitochondria. Microsomes and mitochondria were prepared from the initial 1000 x g supernatant (see Fig. 1). Twenty-five milliliters of this supernatant was sedimented at 10,000 x g for 10 min in a Sorvall RC2-B refrigerated centrifuge. Microsomes were prepared from this supernatant (a) and mitochondria were prepared from this pellet (b).

This supernatant (a) was sedimented at 205,000 x g for 30 min in the 50 rotor of a Beckman Model L2-65B ultracentrifuge. The sedimented microsomal fraction was washed once by suspension and resedimentation in 0.25 M sucrose containing 20 mM Tris-Cl pH 7.5 to remove adsorbed cytoplasmic

proteins (5). The final microsomal pellet was suspended in 2.0 ml of deionized water.

The pellet (b) was washed twice by suspension and resedimentation in 0.25 M sucrose containing 1 mM EGTA at 10,000 x g for 10 min and then suspended in 1 to 3 ml of 0.25 M sucrose and adjusted to 48% sucrose (wt/wt) by the addition of 2.45 M sucrose. This suspension was placed into two SW27 tubes, layered with 40% sucrose (wt/wt) and centrifuged at 131,000 x g for 2 h in a Beckman Model L2-65B ultracentrifuge. The mitochondria were recovered from the 48 to 40% interface, adjusted to 8% sucrose (wt/wt) with deionized water, sedimented at 10,000 x g for 10 min and finally suspended in 2.0 ml of deionized water.

Biochemical enzyme assays. Glucose-6-phosphatase was assayed by a modification of the method of Swanson (26) and de Duve et al. (7). The assay was carried out in a total volume of 1-ml containing 10 mM G-6-P, 100 mM Tris-malate pH 6.6, and enough protein to hydrolyze no more than 30% of the substrate in 20 min at 37 C. Samples of 0.2 ml were withdrawn at appropriate time intervals and added to 0.2 ml of 20% trichloroacetic acid (vol/vol). Inorganic phosphate was analyzed after removal of protein by centrifugation.

5'-Nucleotidase was assayed by the method of Widnell (32). Acid phosphatase was assayed in the same manner as glucose-6-phosphatase except that the incubation contained 100 mM β-glycerolphosphate, 200 mM sodium acetate (pH 5.0) and 0.2% Triton X-100.

Alkaline phosphatase was assayed at room temperature by a modification of the method of De Chatelet et al. (6) in a 3-ml system containing 620 mM 2-amino-2-methyl-1-propanol buffer (pH 10.2), 0.1 mM $MgCl_2$, 15.2 mM

p-nitrophenol-phosphate and 100 µl of sample. The production of p-nitrophenol was followed for 3 min at 420 nm in a Beckman DU recording spectrophotometer. A molar extinction coefficient of 1.62×10^4 was used to determine specific activities.

Glutamate dehydrogenase was assayed in a 3-ml system at room temperature by a modification of the method of Hoek et al. (11). The system contained 50 mM potassium phosphate buffer (pH 7.4), 20 mM L-glutamic acid, 0.4 mM NAD and 0.2% Triton X-100. The oxidation of glutamate was measured on a Beckman DU recording spectrophotometer by the increase of optical density at 340 nm caused by the reduction of NAD. A molar extinction coefficient of 6.20×10^3 was used to determine specific activities.

Malate dehydrogenase was assayed in the same manner as glutamate dehydrogenase except that the oxidation of NADH was followed. The assay was as described in the Sigma Technical Bulletin (24) except that it contained 0.2% Triton X-100.

Kinetic analysis. Plasma membranes were isolated from control (3×10^5 heat-killed S. pneumoniae) and infected (3×10^5 virulent S. pneumoniae) rats 40 h postinoculation. Each preparation was assayed as described for 5'-nucleotidase and alkaline phosphatase at five substrate concentrations for various lengths of time. Under the assay conditions employed both reactions were linear with respect to time and enzyme concentration.

Electron microscopy. Samples to be prepared for electron microscopy were suspended in 100 mM sodium-cacodylate buffer (pH 7.2) containing 10 mM $MgCl_2$ and fixed for 2 h on ice by the addition of equal volumes of 4% glutaraldehyde in the same buffer. Following glutaraldehyde fixation the samples were washed once in buffer by suspension and centrifugation and

then suspended in buffer and left overnight at 0 C. The samples were then fixed on ice in 1% osmium tetroxide (vol/vol) for 3 h, sedimented, washed in 50 mM sodium-cacodylate buffer (pH 7.2) containing 5 mM $MgCl_2$, dehydrated in a series of graded ethanols followed by propylene oxide and finally embedded in Epon-Araldite. Sections prepared with a diamond knife on a Reichert ultramicrotome were stained with uranyl acetate and lead citrate (20) and examined with an Hitachi electron microscope at 75 kv.

RESULTS

Figure 2 shows the mean febrile response of 6 fasted control rats inoculated with 3×10^5 heat-killed S. pneumoniae and 12 fasted infected rats inoculated with the same dose of virulent S. pneumoniae. The normal rectal temperature of these rats varied slightly depending upon the time of the day; temperatures were slightly higher during the night cycle (2:00 A.M. to 2:00 P.M.). Two of the 12 infected rats survived this dose and both of these rats showed an increase of rectal temperature at 40 h and a return to control temperature by 95 h. The mean time to death was 66.1 h with a range of 40 to 95.5 h.

In initial experiments attempted to define optimal conditions for the preparation of plasma membranes with a high yield and low contamination from other organelles, Ray's method (19), modified as described, consistently provided the best results and was therefore used in all subsequent experiments. Since identical results were obtained in perfused or non-perfused livers, membranes were routinely isolated from nonperfused livers. As previously reported (19) the addition of calcium to the homogenization medium resulted in nearly a two-fold increase in the yield of isolated

plasma membranes.

Table 1 shows the percent of total activity for selected enzymes recovered with the plasma membranes from control and infected rats fasted 20 and 40 h. Approximately 10 to 30% of the plasma membrane markers, 5'-nucleotidase and alkaline phosphatase, were recovered with the plasma membrane fraction for control or infected rats fasted for 20 or 40 h. This recovery value is higher than most previously reported figures (8). It appears from Table 1 that more of the marker enzymes for the plasma membrane was recovered from the control and infected animals at 40 h. This may be the result, however, of the higher yield of plasma membranes for these 40-h preparations. It should be noted that plasma membrane yields varied from 1 to 2 mg plasma membrane protein per gram of liver for both control and infected animals at both 20 and 40 h.

Less than 0.433% of the glucose-6-phosphatase, a marker for the endoplasmic reticulum, was recovered with the plasma membranes. Table 1 also demonstrates that less than 0.255% of both markers for mitochondria, glutamate and malate dehydrogenase, and less than 0.103% of the marker for lysosomes, acid phosphatase, were recovered with the isolated plasma membrane fraction.

It should be noted that the stability of glutamate dehydrogenase under our homogenization and assay conditions was determined since reports have appeared stating that this enzyme is unstable (23). Glutamate dehydrogenase activity was stable under the conditions employed here.

Table 2 shows that these plasma membrane preparations contained approximately 12% contamination by microsomes and mitochondria, as judged from the enzyme markers for these fractions. Washing isolated plasma membranes in 150 mM NaCl, 10 mM EGTA, or 20 mM Tris-Cl (pH 7.5) did not

improve the purity. Differential centrifugation was also employed in an attempt to remove contamination. Plasma membranes isolated from the sucrose gradient (Fig. 1) were adjusted to 8% sucrose (wt/wt) and sedimented at either 1000 or 10,000 x g for 10 min. Essentially the same amount of activity for all marker enzymes sedimented under both forces.

Slightly purer preparations could be obtained, however, when isolated plasma membranes were washed in 20 mM Tris-Cl pH 7.5, layered on a continuous sucrose gradient (31 to 41% wt/wt), and centrifuged overnight. It was difficult to assess the biochemical contamination of these preparations, however, since certain of the enzyme markers employed lost a significant amount of activity following overnight centrifugation. It was therefore decided to use the membranes prepared as described for all subsequent studies.

The effects of S. pneumoniae infection on the specific activities of selected liver enzymes in both the homogenate and isolated cell fractions are shown in Table 3. 5'-Nucleotidase and alkaline phosphatase were chosen as markers for plasma membranes (10, 28); however, cytochemical experiments have shown that 5'-nucleotidase is also localized in microsomes (31). The specific activity of this enzyme showed no change 20 h following infection in either the homogenate or any of the cell fractions studied. At 40 h, however, the specific activity of 5'-nucleotidase decreased significantly in both homogenate and all cell fractions. On the other hand, the specific activity of alkaline phosphatase in the plasma membrane fraction increased significantly 20 and 40 h following infection. At 40 h it was also increased in the homogenate and microsomal fractions. It has been shown that this enzyme is inducible following bacterial infections (14, 29). The mitochondrial markers, malate and glutamate dehydrogenase (16, 25), were unchanged

following infection. It should be noted, however, that malate dehydrogenase is also found in the soluble fraction of the cell (16).

The specific activity of glucose-6-phosphatase (endoplasmic reticulum) decreased significantly in the homogenate and in the microsomal and plasma membrane fractions at both 20 and 40 h postinfection. These results are in agreement with a previously published report (3). The specific activity of acid phosphatase (lysosomes) decreased significantly in the homogenate and plasma membrane fraction at 40 h. It is also important to note (Table 3) that infection did not significantly alter the yields of any fraction studied.

Fig. 3 shows the kinetic analysis of 5'-nucleotidase and Fig. 4 of alkaline phosphatase in isolated plasma membranes from both control and infected rats. These results show that S. pneumoniae infection did not significantly alter the K_m of either 5'-nucleotidase (control and infected K_m = 1.29 mM) or alkaline phosphatase (control and infected K_m = 0.330 mM). As could be predicted from Table 3 the V_{max} for both enzymes was altered following infection. The V_{max} for 5'-nucleotidase decreased from 13.9 to 10.5 µmol whereas the V_{max} for alkaline phosphatase increased from 30.1 to 80.0 nmol.

Figure 5 shows electron micrographs of plasma membranes isolated from control and infected animals. Both preparations appeared similar except that the preparations from the latter seemed slightly more compact. From scanning thin sections, it appeared that the contamination from mitochondria, lysosomes, and rough microsomes was minimal; only very rarely were these organelles observed. As mentioned, slightly purer preparations could be obtained by overnight centrifugation of the isolated plasma membranes in a continuous sucrose gradient. These preparations are also shown in Fig. 5.

DISCUSSION

Isolation and partial characterization of relatively pure plasma membranes, microsomes and mitochondria from control and infected rats revealed evidence for decreased plasma membrane 5'-nucleotidase but increased alkaline phosphatase. A decrease in microsomal glucose-6-phosphatase was also observed but the two marker enzymes for the mitochondria, glutamate and malate dehydrogenase, showed no change.

The mechanism(s) responsible, however, for the alteration in the specific activity of 5'-nucleotidase and alkaline phosphatase in the plasma membrane following infection have not been determined. One possibility is that infection may alter the synthesis or degradation of these enzymes or other molecules serving as activators or inhibitors. The observed alterations do not appear to be the result of altered kinetic properties of the enzymes since the K_m of both 5'-nucleotidase and alkaline phosphatase were the same in control and infected rats. The increased V_{max} for alkaline phosphatase suggests either increased enzyme synthesis, decreased degradation, or activation of preexisting enzyme whereas the decreased V_{max} for 5'-nucleotidase suggest either decreased synthesis, increased degradation, or masking of existing enzyme. While it is possible that 5'-nucleotidase is being synthesized but not incorporated into the plasma membrane, it appears unlikely since the specific activity of the enzyme is decreased in the homogenate and microsomal fraction as well as in the plasma membrane fraction 40 h postinfection. If the enzyme were synthesized but not incorporated into the plasma membrane, then one would expect to find an increase in the homogenate and possibly in the microsomal fraction but a decrease in the plasma membrane fraction.

The observed decrease in glucose-6-phosphatase activity is not merely the result of glycogen depletion which occurs during fasting. Nor does it appear to be the result of altered kinetic behavior since preliminary experiments suggest that the K_m of glucose-6-phosphatase for glucose-6-phosphate in the microsomal fraction is the same for control and infected animals.

These results could also be explained by a change in membrane properties following infection such that they behave differently in the isolation procedure employed. However, since both the total yield of plasma membranes and the percent contamination by other cell organelles was similar following infection, it is doubtful that these results are an artifact of the method.

It should also be noted that these preparations are heterogeneous since liver contains both parenchymal cells and reticuloendothelial (Kupffer) cells. Suspensions of pure hepatic parenchymal cells may be preferable; however, techniques required to produce homogeneous parenchymal cell suspensions involve the use of collagenase and hyaluronidase (12), both of which may seriously alter the plasma membrane. Since the liver is heterogeneous with respect to cell type, the observed enzyme alterations could then be due to changes in parenchymal or Kupffer cells, or both. It is possible that the alkaline phosphatase increase represents a proliferation in the phagocytic Kupffer cell which may then serve to enhance host resistance. At present there is no evidence to support this idea.

The reasons for the alterations in organelle enzyme profile following S. pneumoniae infection have not been determined. It may represent a purposeful and selective synthesis of certain proteins (enzymes) of benefit to the host during infection at the expense of other enzymes momentarily dispensable.

Experiments are in progress to determine the significance of these alterations and what role the plasma membrane plays in maintaining cell homeostasis following S. pneumoniae infection.

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LITERATURE CITED

1. Ames, B. N., and D. T. Dubin. 1960. The role of polyamines in the neutralization of bacteriophage deoxyribonucleic acid. J. Biol. Chem. 235:769-775.

2. Bock, R. M., N.-S. Ling, S. A. Morell, and S. H. Lipton. 1956. Ultraviolet absorption spectra of adenosine-5'-triphosphate and related 5'-ribonucleotides. Arch. Biochem. Biophys. 62:253-264.

3. Canonico, P. G., J. D. White, and M. C. Powanda. 1975. Peroxisome depletion in rat liver during pneumococcal sepsis. Lab. Invest. 33:147-149.

4. Chen, P. S., T. Y. Toribara, and H. Warner. 1956. Microdetermination of phosphorus. Anal. Chem. 28:1756-1758.

5. Dallner, G., P. Siekevitz, and G. E. Palade. 1966. Biogenesis of endoplasmic reticulum membranes. II. Synthesis of constitutive microsomal enzymes in developing rat hepatocyte. J. Cell Biol. 30:97-117.

6. DeChatelet, L. R., and M. R. Cooper. 1970. A modified procedure for the determination of leukocyte alkaline phosphatase. Biochem. Med. 4:61-68.

7. de Duve, C., J. Berthet, H. G. Hers, and L. Dupret. 1949. Le système hexose-phosphatasique. I. Existence d'une glucose-6-phosphatase spécifique dans le foie. Bull. Soc. Chem. Biol. 31:1242-1253.

8. DePierre, J. W., and M. L. Karnovsky. 1973. Plasma membranes of mammalian cells. A review of methods for their characterization and isolation. J. Cell Biol. 56:275-303.

9. Dubois-Dalcq, M., and T. S. Reese. 1975. Structural changes in the membrane of Vero cells infected with a paramyxovirus. J. Cell Biol. 67:551-565.

10. Emmelot, P., and C. J. Bos. 1966. Studies on plasma membranes. II. K^+-dependent p-nitrophenyl phosphatase activity of plasma membranes isolated from rat liver. Biochim. Biophys. Acta 121:375-385.

11. Hoek, J. B., L. Ernster, E. J. De Haan, and J. M. Tager. 1974. The nicotinamide nucleotide specificity of glutamate dehydrogenase in intact rat-liver mitochondria. Biochim. Biophys. Acta 333:546-559.

12. Howard, R. B., J. C. Lee, and L. A. Pesch. 1973. The fine structure, potassium content, and respiratory activity of isolated rat liver parenchymal cells prepared by improved enyzmatic techniques. J. Cell Biol. 57:642-658.

13. Kaaden, O. R., and B. Dietzschold. 1974. Alterations of the immunological specificity of plasma membranes from cells infected with Marek's disease and turkey herpes viruses. J. Gen. Virol. 25:1-10.

14. Kaplow, L. S. 1968. Leukocyte alkaline phosphatase-cytochemistry: applications and methods. Ann. N.Y. Acad. Sci. 155:911-947.

15. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.

16. Marco, R., J. Sebastian, and A. Sols. 1969. Localization of the enzymes of the oxalacetate metabolic cross-roads in rat liver mitochondria. Biochem. Biophys. Res. Commun. 34:725-730.

17. Ninjoor, V., and U. S. Kumta. 1974. Sedimentation and osmofragility characteristics of rat liver lysosomes. Alterations caused by feeding protein-free diet to adult rats. Indian J. Biochem. Biophys. 11:221-226.

18. Pekarek, R. S., R. W. Wannemacher, Jr., and W. R. Beisel. 1972. The effect of leukocytic endogenous mediator (LEM) on tissue distribution of zinc and iron. Proc. Soc. Exp. Biol. Med. 140:685-688.

19. Ray, T. K. 1970. A modified method for the isolation of the plasma membrane from rat liver. Biochim. Biophys. Acta 196:1-9.

20. Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17:208-212.

21. Rocha, D. M., F. Santeusanio, G. R. Faloona, and R. H. Unger. 1973. Abnormal pancreatic alpha-cell function in bacterial infections. N. Engl. J. Med. 288:700-703.

22. Ryan, N. T., G. I. Blackburn, and G. H. A. Clowes, Jr. 1974. Differential tissue sensitivity to elevated endogenous insulin levels during experimental peritonitis in rats. Metabolism 23:1081-1089.

23. Salinas, M., R. Wallace, and S. Grisolia. 1974. Comparative studies in vivo and in vitro of rat-liver enzymes. Eur. J. Biochem. 44:375-381.

24. Sigma Technical Bulletin No. 340-UV. 1974. Sigma Chemical Co., St. Louis, Missouri.

25. Sottocasa, G. L., B. Kuylenstierna, L. Ernster, and A. Bergstrand. 1967. Separation and some enzymatic properties of the inner and

outer membranes of rat liver mitochondria, p. 448-463. In R. W. Estabrook and M. E. Pullman (eds.), Method in Enzymology, Vol. 10, Academic Press, New York.

26. Swanson, M. A. 1950. Phosphatases of liver. I. Glucose-6-phosphatase. J. Biol. Chem. 184:647-659.

27. Takacs, B. J., and J. P. Rosenbusch. 1975. Modification of Escherichia coli membranes in the prereplicative phase of phage T4 infection. J. Biol. Chem. 250:2339-2350.

28. Wachstein, M., and E. Meisel. 1957. Histochemistry of hepatic phosphatases at physiological pH. Am. J. Clin. Pathol. 27:13-23.

29. Wachstein, M. 1946. Alkaline phosphatase activity in normal and abnormal human blood and bone marrow cells. J. Lab. Clin. Med. 31:1-17.

30. Wannemacher, R. W., Jr., R. S. Pekarek, and W. R. Beisel. 1972. Mediator of hepatic amino acid flux in infected rats. Proc. Soc. Exp. Biol. Med. 139:128-132.

31. Widnell, C. C. 1972. Cytochemical localization of 5'-nucleotidase in subcellular fractions isolated from rat liver. I. The origin of 5'-nucleotidase activity in microsomes. J. Cell Biol. 52:542-558.

32. Widnell, C. C. 1974. Purification of rat liver 5'-nucleotidase as a complex with sphingomyelin, p. 368-374. In S. Fleischer and L. Packer (eds.), Methods in Enzymology, Vol. 32, Academic Press, New York.

33. Zenser, T. V., F. R. DeRubertis, D. T. George, and E. J. Rayfield. 1974. Infection-induced hyperglucagonemia and altered hepatic response to glucagon in the rat. Am. J. Physiol. 227:1299-1305.

TABLE 1. TOTAL HOMOGENATE ACTIVITY OF SELECTED ENZYMES RECOVERED WITH PLASMA MEMBRANES ISOLATED FROM CONTROL AND INFECTED RATS FASTED 20 AND 40 h

Enzyme	% Activity[a]			
	20 h		40 h	
	Control	Infected	Control	Infected
5'-Nucleotidase	10.7	13.2	20.2	30.1
Alkaline phosphatase	19.8	29.7	19.0	25.4
Glucose-6-phosphatase	0.263	0.316	0.433	0.424
Glutamate dehydrogenase	0.194	0.071	0.255	0.351
Malate dehydrogenase	0.133	0.141	0.200	0.260
Acid phosphatase	0.103	0.074	0.100	0.093

[a] $\% = \frac{\text{specific activity of enzyme in plasma membranes X plasma membrane yield}}{\text{specific activity of enzyme in homogenate X homogenate yield}} \times 100$

Data from Table 3 were used to obtain these values.

TABLE 2. BIOCHEMICAL ASSESSMENT OF CONTAMINATION OF ISOLATED PLASMA MEMBRANES BY OTHER CELL ORGANELLES.

Cell Fraction	Specific Activity[a]			
	Glucose-6-phosphatase[b]		Glutamate dehydrogenase[c]	
	Control	Infected	Control	Infected
Plasma membrane (PM)	1.00 ± 0.080	0.600 ± 0.050	0.004 ± 0.002	0.005 ± 0.002
Microsomes (M)	8.30 ± 0.197	4.99 ± 0.395	--	--
Mitochondria (MC)	--	--	0.032 ± 0.003	0.031 ± 0.002
Lysosomes (L)	--	--	--	---
Ratio x 100%	PM/M = 12.0	PM/M = 12.0	PM/MC = 12.5	PM/MC = 16.1
	Malate dehydrogenase[c]		Acid phosphatase[b]	
Plasma membrane (PM)	0.280 ± 0.044	0.285 ± 0.022	0.117 ± 0.022	0.065 ± 0.006
Microsomes (M)	--	--	--	--
Mitochondria (MC)	2.20 ± 0.138	1.97 ± 0.047	--	--
Lysosomes (L)	--	--	11.1[d]	ND[e]
Ratio x 100%	PM/MC = 12.7	PM/MC = 14.5	PM/L = 1.10	--

[a] Mean of 6 rats ± standard error of the mean

[b] µmol Pi released/20 min per mg protein.

[c] µmol formed/min per mg.

[d] (value from ref. 17).

[e] ND = not determined.

TABLE 3. SPECIFIC ACTIVITY OF SELECTED LIVER ENZYMES IN HOMOGENATE AND ISOLATED CELL FRACTIONS OF INFECTED AND CONTROL RATS

Enzyme	Specific Activity[a]			
	20 h		40 h	
	Controls	Infected	Controls	Infected
HOMOGENATE				
5'-Nucleotidase[b]	1.60 ± 0.113	1.42 ± 0.060	1.40 ± 0.079	0.868 ± 0.103[f]
Alkaline phosphatase[c]	0.666 ± 0.055	0.853 ± 0.200	1.13 ± 0.124	2.14 ± 0.438[f]
Malate dehydrogenase[d]	1.25 ± 0.056	1.04 ± 0.016[f]	1.29 ± 0.055	1.15 ± 0.048
Glutamate dehydrogenase[d]	0.009 ± 0.0005	0.008 ± 0.0004	0.016 ± 0.0005	0.015 ± 0.0007
Glucose-6-phosphatase[b]	2.24 ± 0.090	1.79 ± 0.079[g]	2.16 ± 0.067	1.49 ± 0.070[g]
Acid phosphatase[b]	0.706 ± 0.142	0.744 ± 0.148	1.05 ± 0.072	0.735 ± 0.050[f]
PLASMA MEMBRANES				
5'-Nucleotidase[b]	29.2 ± 2.12	32.8 ± 2.60	30.3 ± 1.48	24.8 ± 1.68[f]
Alkaline phosphatase[c]	22.5 ± 1.30	44.5 ± 6.34[f]	23.9 ± 2.38	51.6 ± 10.0[f]
Malate dehydrogenase[d]	0.285 ± 0.026	0.258 ± 0.020	0.286 ± 0.044	0.285 ± 0.022
Glutamate dehydrogenase[d]	0.003 ± 0.000	0.001 ± 0.000	0.004 ± 0.002	0.005 ± 0.002
Glucose-6-phosphatase[b]	1.46 ± 0.048	0.993 ± 0.052[g]	1.00 ± 0.080	0.600 ± 0.050[g]
Acid phosphatase[b]	0.124 ± 0.012	0.097 ± 0.012	0.117 ± 0.022	0.065 ± 0.006[f]
MICROSOMES				
5'-Nucleotidase[b]	2.79 ± 0.134	2.63 ± 0.095	3.23 ± 0.207	2.01 ± 0.127[g]
Alkaline phosphatase[c]	1.41 ± 0.231	1.99 ± 0.321	1.58 ± 0.243	3.27 ± 0.531[f]
Malate dehydrogenase[d]	0.053 ± 0.009	0.048 ± 0.007	0.069 ± 0.007	0.061 ± 0.008
Glutamate dehydrogenase[d]	0.001 ± 0.0002	0.001 ± 0.0001	0.002 ± 0.0002	0.001 ± 0.0005[f]
Glucose-6-phosphatase[b]	7.57 ± 0.292	5.70 ± 0.369[g]	8.30 ± 0.197	4.99 ± 0.395[g]

TABLE 3. Continued ...

MITOCHONDRIA				
5'-Nucleotidase[b]	0.286 ± 0.055	N.D.[e]	0.286 ± 0.055	0.137 ± 0.010[f]
Alkaline phosphatase[c]	Not detectable	Not detectable	Not detectable	Not detectable
Malate dehydrogenase[d]	N.D.	N.D.	2.20 ± 0.138	1.97 ± 0.047
Glutamate dehydrogenase[d]	N.D.	N.D.	0.032 ± 0.003	0.031 ± 0.002
Glucose-6-phosphatase[b]	N.D.	N.D.	0.401 ± 0.064	0.458 ± 0.033
YIELD mg/g liver				
Homogenate	170 ± 6.43	175 ± 1.20	185 ± 12.6	173 ± 19.1
Plasma membranes	0.994 ± 0.067	0.997 ± 0.88	1.73 ± 0.250	1.82 ± 0.240
Microsomes	15.5 ± 0.840	15.5 ± 0.48	17.7 ± 0.910	16.5 ± 0.570
Mitochondria	N.D.	N.D.	6.56 ± 0.660	8.58 ± 0.980

[a] Mean of 6 rats ± standard error of the mean.

[b] µmol Pi released/20 min per mg protein at 37 C.

[c] nmol formed/min per mg protein.

[d] µmol formed/min per mg protein.

[e] N.D. = not determined.

[f] $P < 0.05$

[g] $P < 0.005$

LEGENDS TO FIGURES

Fig. 1. Flow diagram for preparation of plasma membranes, mitochondria, and microsomes.

Fig. 2. Rectal temperatures of control and S. pneumoniae-infected rats. Six control (Δ - 3×10^5 heat-killed) and 12 infected (O - 3×10^5 virulent) rats were inoculated subcutaneously with S. pneumoniae

Fig. 3. Kinetic analysis of 5'-nucleotidase in plasma membranes isolated from control and infected rats. Values represent the mean ± SEM of 6 control (Δ) or 6 infected (O) rats inoculated with either 3×10^5 heat-killed (Δ) or virulent (O) S. pneumoniae.

Fig. 4. Kinetic analysis of alkaline phosphatase in plasma membranes isolated from control and infected rats. Values represent the mean ± SEM of 6 control (Δ) or 6 infected (O) rats inoculated with either 3×10^5 heat-killed (Δ) or virulent (O) S. pneumoniae.

Fig. 5. Electron micrographs of purified plasma membranes.

A. Plasma membranes isolated as described from control rats. X 18,000.

B. Same as (A) but from infected rats.

C. Same as (A) but further purified by centrifugation in a 31 to 41% (wt/wt) sucrose gradient. X 18,000.

D. Same as (C) but for infected rats.

FIGURE 1

PREPARATION OF PLASMA MEMBRANES, MITOCHONDRIA, AND MICROSOMES

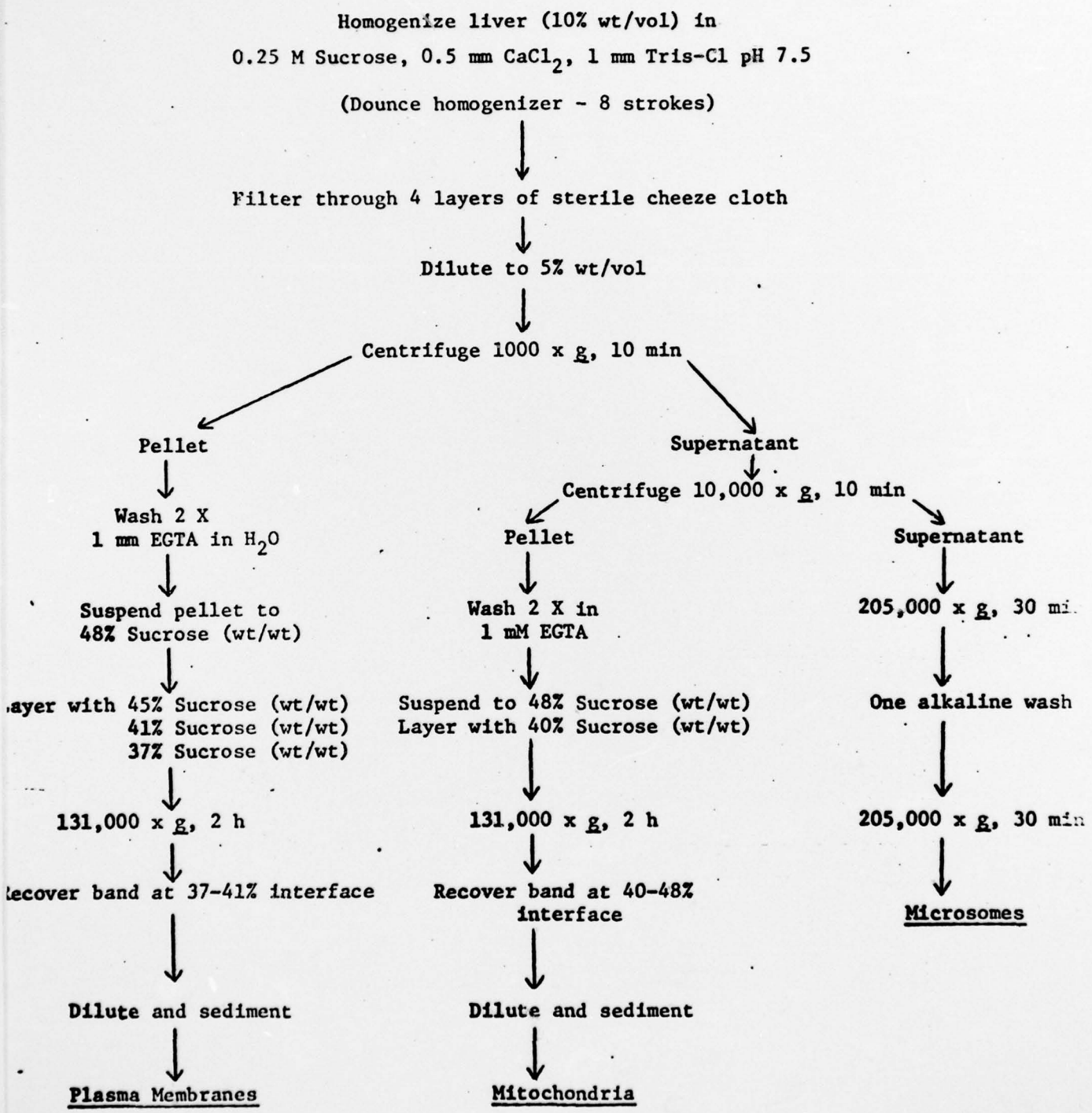


Fig. 2

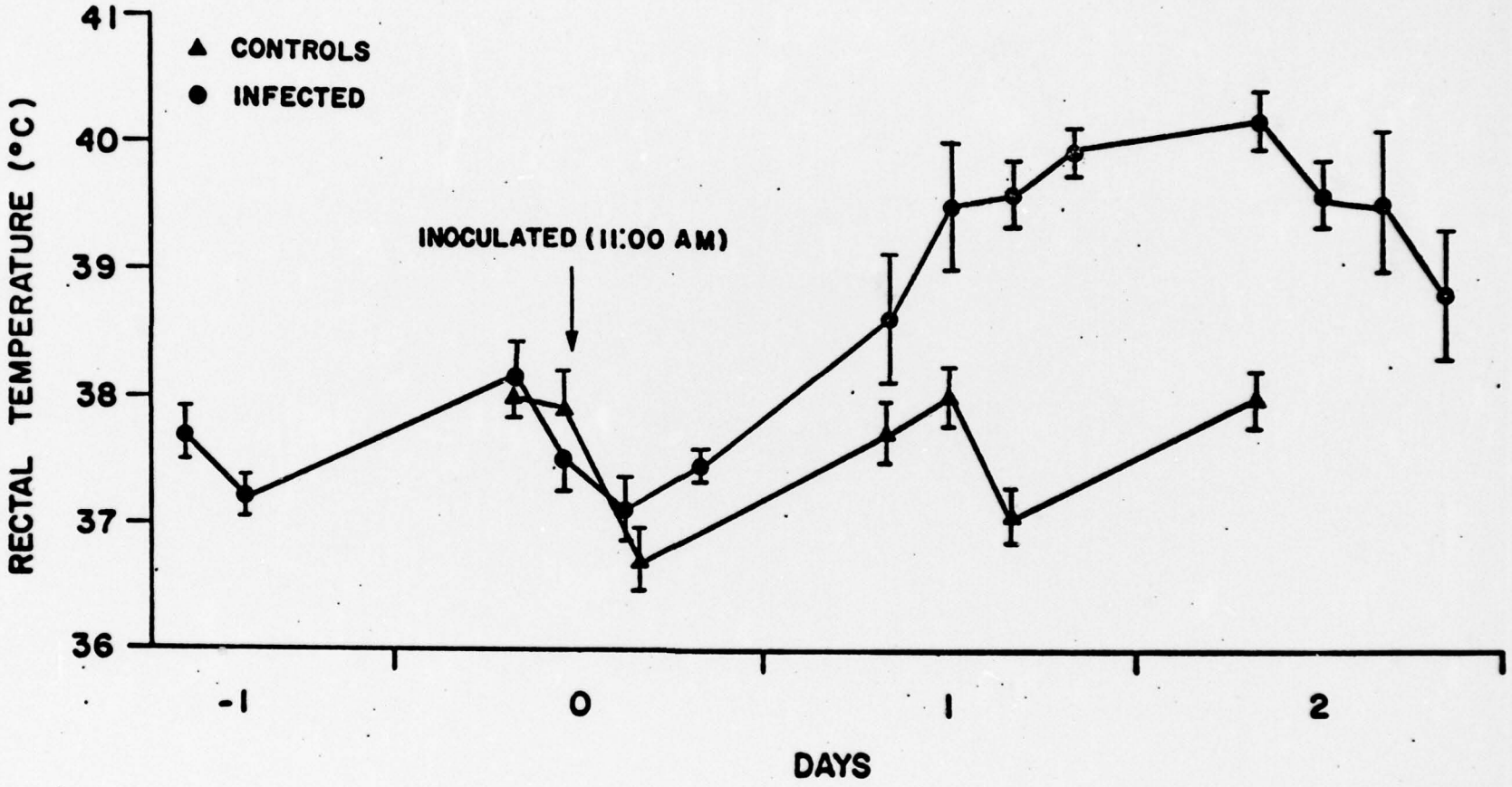

41
40
39
38
37
36
RECTAL TEMPERATURE (°C)
▲ CONTROLS
● INFECTED
INOCULATED (11:00 AM)
-1
0
1
2
DAYS

Fig 3.

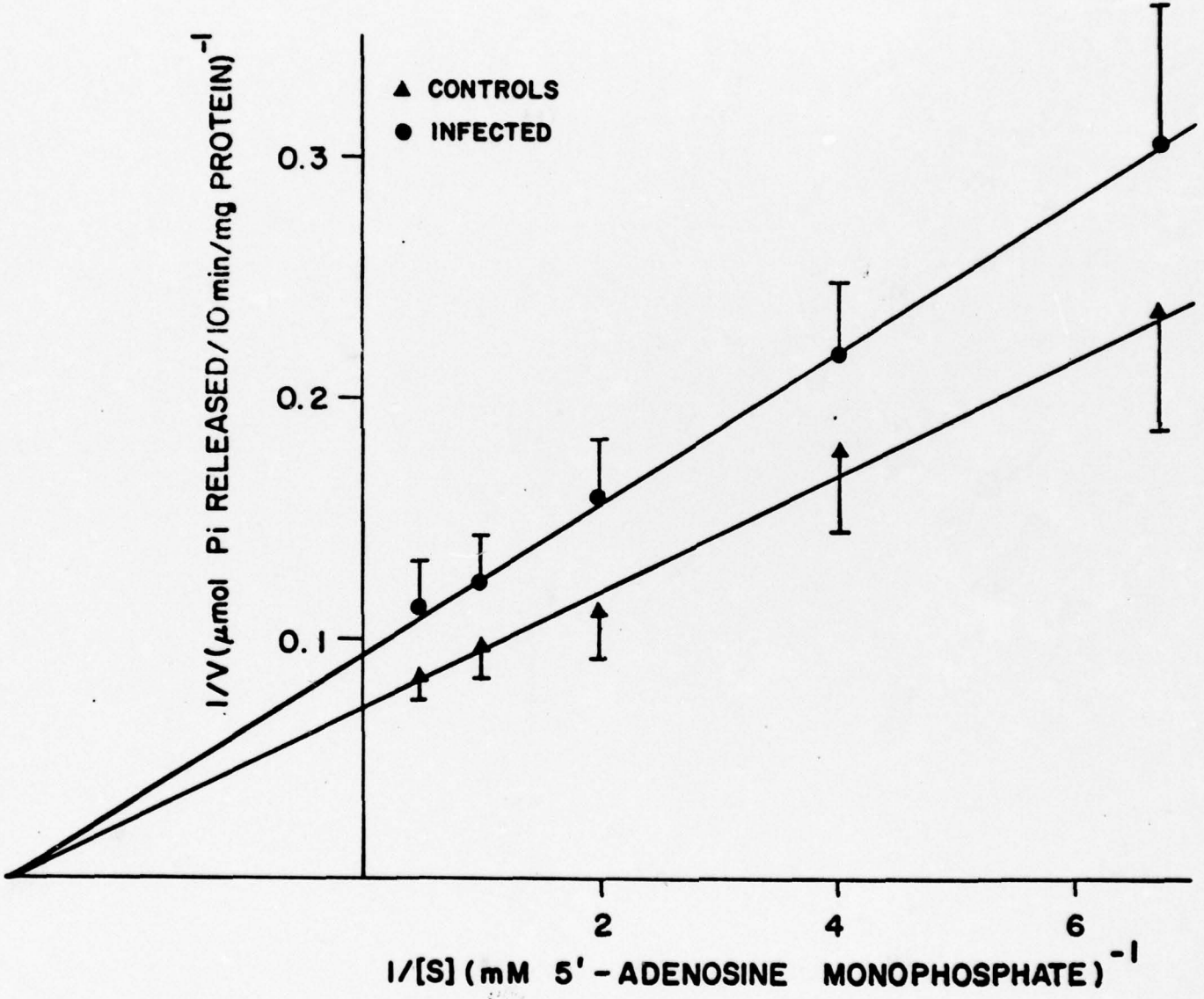
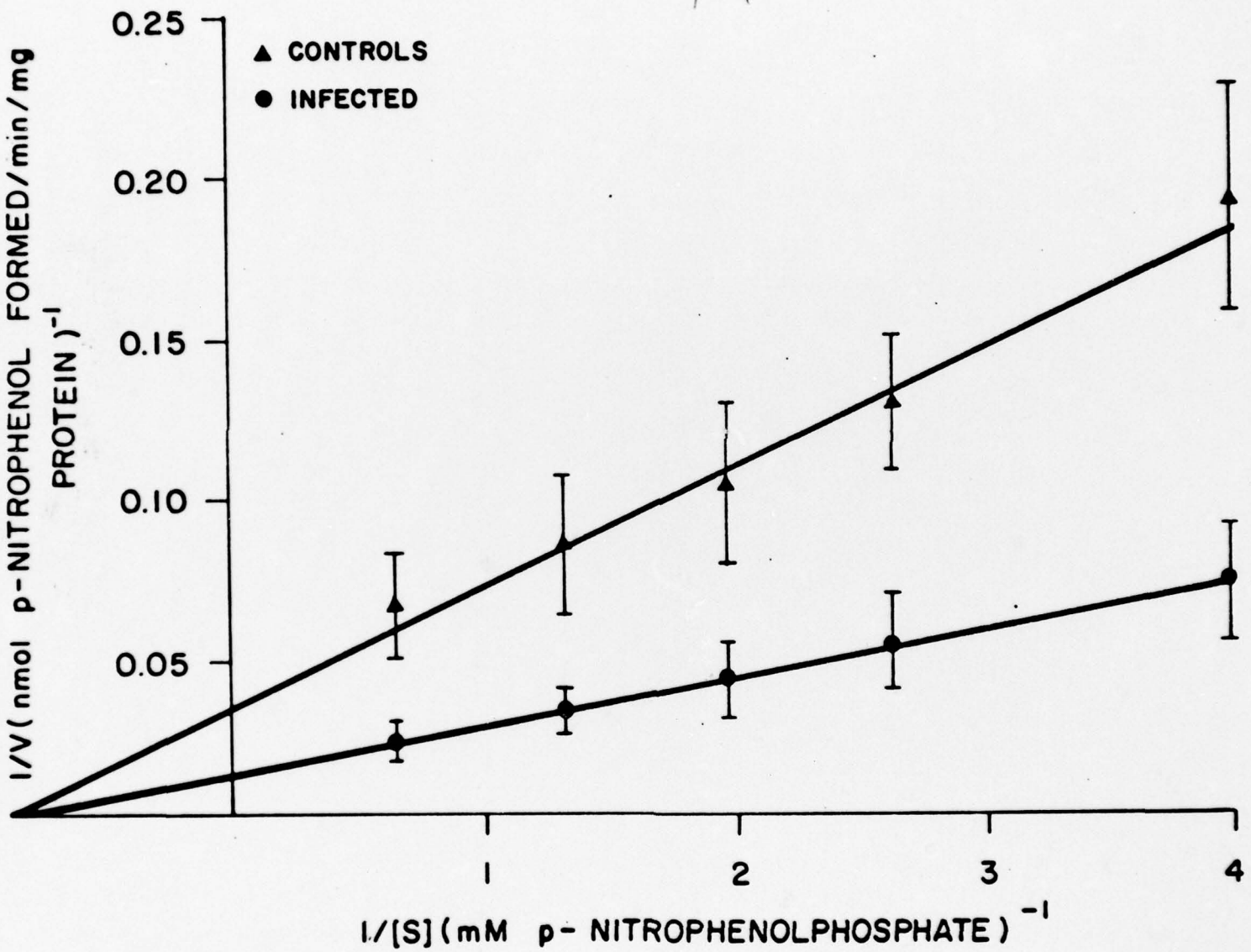
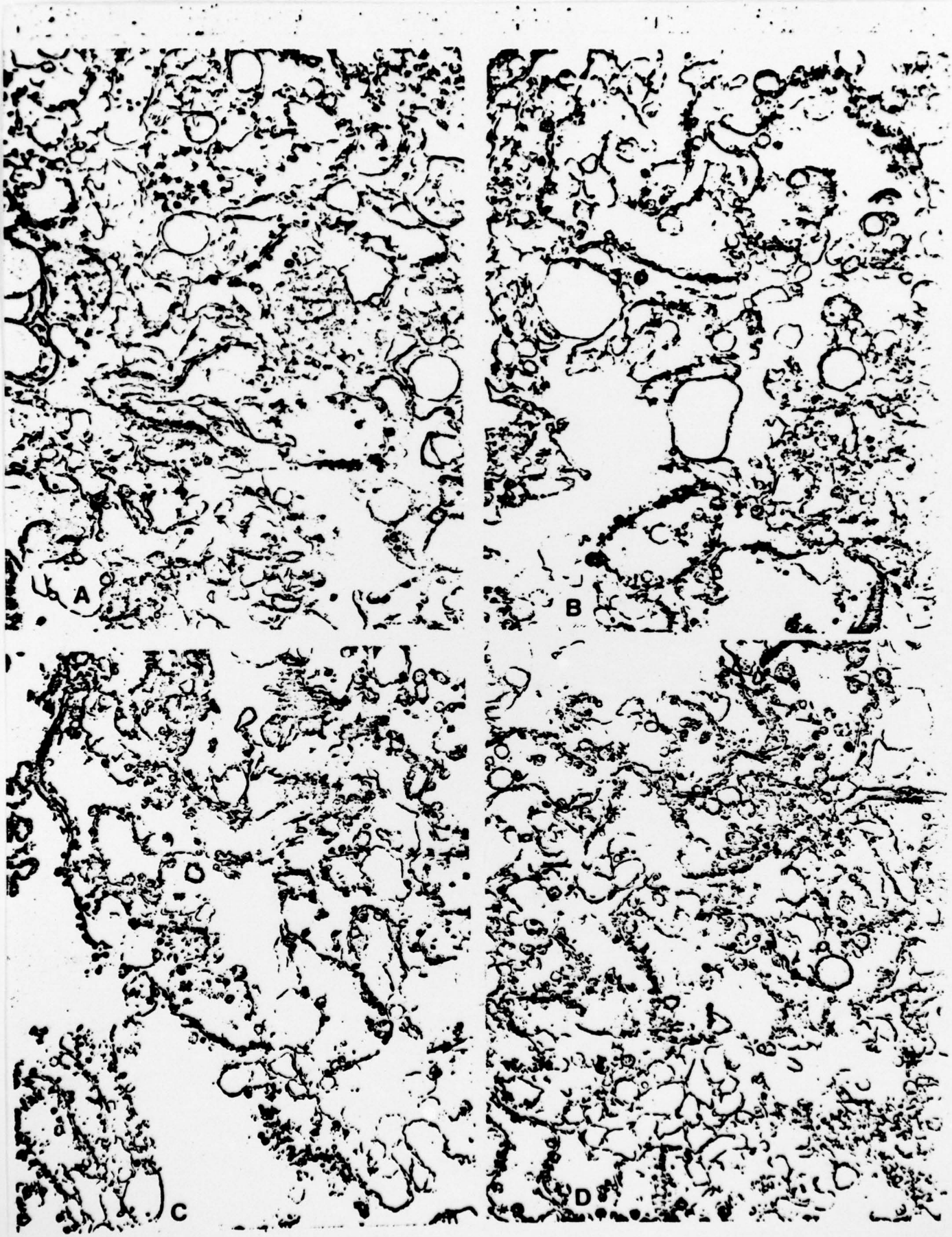

▲ CONTROLS
● INFECTED
0.3
0.2
0.1
1/V(μmol Pi RELEASED/10 min/mg PROTEIN)$^{-1}$
2
4
6
1/[S] (mM 5'-ADENOSINE MONOPHOSPHATE)$^{-1}$

Fig. 4


0.25
0.20
0.15
0.10
0.05
▲ CONTROLS
● INFECTED
1/V(nmol p-NITROPHENOL FORMED/min/mg PROTEIN)$^{-1}$
1
2
3
4
1/[S] (mM p- NITROPHENOLPHOSPHATE)$^{-1}$


A
B
C
D